Remarks/Argument

By the present amendment, claims 1-2, 5, 7, 21-23, and 38 have been amended. Support for amended claims 1, 23, and 38 can be found at at least p. 5, ll. 20-23, p. 6, ll. 11-14, and p. 12, ll. 12-15 of the present application.

Below is a discussion of the 35 U.S.C. §112, second paragraph, rejection of claim 1, the 35 U.S.C. §112, first paragraph, rejection of claims 1-2, 5, 7, 21-23 and 37-38, and the 35 U.S.C. §103(a) rejection of claims 1-2, 5, 7, 21-23, and 37-38.

1. 35 U.S.C. §112, second paragraph, rejection of claim 1.

Claim 1 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Office Action argues that it is unclear what the phrase "other than IGF/vitronectin (VN) synthetic chimera" means.

By the present amendment, claim 1 has been amended to more clearly define what is meant by the phrase "other than IGF/vitronectin (VN) synthetic chimera". More particularly, claim 1 has been amended to recite that "a synthetic chimeric protein…comprises at least one Insulin-like Growth Factor (IGF), and an α_v integrin-receptor binding vitronectin (VN) fragment that does not comprise a heparin binding domain". Additionally, the specification of the present application clearly recites what is meant by the term "synthetic" (*see*, *e.g.*, p. 6, ll. 11-15). Furthermore, examples of what the term "synthetic chimeric protein" can encompass are described on p. 11, line 17 to p. 12, line 28 of the present application.

Accordingly, Applicants respectively submit that it is clear from the specification of the present application what the phrase "other than IGF/vitronectin

(VN) synthetic chimera" means, and request that the 35 U.S.C. §112, second paragraph, rejection of claim 1 be withdrawn.

2. 35 U.S.C. §112, first paragraph, rejection of claims 1-2, 5, 7, 21-23 and 37-38.

Claims 1-2, 5, 7, 21-23, and 37-38 were rejected under 35 U.S.C. §112, first paragraph, as (1) failing to comply with the written description requirement and (2) containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

By the present amendment, the negative limitation reciting "other than as an IGF-I/vitronectin (VN) synthetic chimera" has been deleted from claim 1.

Accordingly, Applicants respectively submit that the 35 U.S.C. §112, first paragraph, rejection of claim 1 is rendered moot. Additionally, Applicants respectively request that the 35 U.S.C. §112, first paragraph, rejection of claims 2, 5, 7, 21-23 and 37-38, which depend either directly or indirectly from amended claim 1, be withdrawn.

3. 35 U.S.C. §103(a) rejection of claims 1-2, 5, 7, 21-23, and 37-38.

Claims 1-2, 5, 7, 21-23, and 37-38 were rejected under 35 U.S.C. §103(a) as being unpatentable over PCT Publication No. WO 02/24219 to Upton *et al.* (hereinafter, "Upton"), U.S. Patent No. 5,830,504 to Vuori *et al.* (hereinafter, "Vuori"), and U.S. Patent No. 5,407,913 to Sommer *et al.* (hereinafter, "Sommer") in view of Klemke *et al.*, *J Cell Bio.* 127:859-866, 1994 (hereinafter, "Klemke") and Nam *et al.*, *Endocrinology* 143(1):30-36, 2002 (hereinafter, "Nam").

By the present amendment, claim 1 has been amended to more particularly point out and distinctly claim the present invention and to better define the present invention over Upton, Vuori, and Sommer in view of Klemke and Nam. More particularly, claim 1 has been amended to further recite a mammalian cell culture medium that (i) comprises a synthetic IGF/VN fragment chimeric protein that does not comprise an IGFBP, and (ii) is capable of supporting cell growth in low or no serum.

For the reasons set forth below, Applicants respectively submit that amended claim 1 patentable over Upton, Vuori, and Sommer in view of Klemke and Nam because: (1) the combination of references does not teach or disclose all the feature recited in amended claim 1; and (2) one having ordinary skill in the art at the time of the present invention would not have had a rational basis to combine Upton, Vuori, and Sommer with Klemke and Nam to arrive at the subject matter of amended claim 1.

I. Upton

The Office Action argues that Upton teaches a mammalian cell culture system of human keratinocytes, wherein the medium comprises (i) IGF-I, (ii) vitronectin, and (iii) an absence of serum. In doing so, the Office Action appears to have not appreciated that Upton actually teaches a serum-containing medium, which is unlike the medium recited in amended claim 1. For instance, the portion of Upton relied upon by the Office Action as teaching a serum-free medium (*i.e.*, pp. 28-29) does not actually teach a medium suitable for cell culture because the only serum-free medium (if any) taught by Upton is a buffering solution (*i.e.*, HEPES or TES) suitable

for optimizing interactions between radio-labeled ligands (*i.e.*, IGF-I[^{125}]) and their cell surface receptors. One skilled in the art at the time of the present invention would have understood that this buffering solution would not be suitable for long-term cultivation of delicate primary epithelial cells, including primary keratinocytes.

If anything, Upton actually teaches away from the present invention as Upton teaches that the binding of IGF-I to VN was examined "in the presence of IGFBPs" (p. 28, ll. 21-23) and that "IGFBPs are required to mediate the binding of IGF-I to VN (p. 29, ll. 4-5). This is in contrast to the presently claimed invention, which teaches a mammalian cell culture medium that (i) comprises a synthetic IGF/VN fragment chimeric protein that does not comprise an IGFBP, and (ii) supports cell migration and growth in the absence of serum.

A. Upton et al., Comparative Biochemistry and Physiology Part B, 121:35-41, 1998 (hereinafter, "Upton 1998")

In support of the argument that Upton teaches a serum-free medium suitable for culturing keratinocytes, the Office Action incorrectly refers to Upton 1998, even though Example 4 of Upton directs the reader to Upton *et al.*, *Endocrinology* 140:2928-2931, 1999 (hereinafter, "Upton 1999"). In further support of the instant rejection, the Office Action also makes reference to the "2.3.2 IGF Binding Studies" section on p. 37, 2nd column of Upton 1998.

Applicants respectively submit that the binding assay medium disclosed by Upton 1998 was only used as a buffering solution to optimize binding of radio-labeled ligands to cell-surface receptors and, hence, would not be suitable for cell culture. Furthermore, the binding experiments described by Upton 1998 were

undertaken using a method and a serum-containing HEPES-binding buffer previously disclosed by Ross *et al.*, *Biochem Journal* 258:267-272, 1989 (hereinafter, "Ross"). For support, Applicants wish to point out the "IGF Binding to Cell Receptors" section on p. 268, 1st column of Ross, which teaches a HEPES buffer comprising 0.5% Bovine Serum Albumin (emphasis added). Applicants also note that HEPES is a zwitterion buffer suitable for modulating the pH of a medium and not designed to support cell growth.

Moreover, in contrast to the serum-free mammalian cell culture medium recited in amended claim 1, which does not comprise an IGFBP and is suitable for cell culture, the medium disclosed by Ross and used by Upton 1998 is a serum-containing medium that would not be suitable for cell culture.

B. Upton 1999, Ballard et al., Biochemical Journal 249:721-726, 1988 (hereinafter, "Ballard 1988), and Ballard et al., Biochemical Journal 233:223-230, 1986 (hereinafter, "Ballard 1986")

In view of the Office Action's incorrect citation of Upton 1998 instead of Upton 1999, Applicants will refer to the relevant sections of Upton 1999 (and references cited therein) to avoid having to address these citations at a later time.

The "Binding Assays" section described by Upton 1999 (p. 2929, 1st column) refers to a serum-containing HEPES-and TES-binding media previously used by Ballard 1988, and originally described by Ballard 1996. For support, Applicants wish to point out the "IGF Binding Experiments" sections on p. 722, 1st column of Ballard 1988, and on p. 224, 2nd column of Ballard 1986. Applicants note that the primary purpose of the HEPES- and TES-binding buffers described by Ballard 1986 and Ballard 1988 was to control the pH of the buffers (*i.e.*, reduce acidity) and optimize

the binding of radio-labeled ligands to their cell surface receptors. Thus, in contrast to the presently claimed invention, which recites a serum-free medium that is suitable for long-term cell cultivation, Upton 1999 teaches a serum-containing medium that would not be suitable for cell culture and is not designed to support cell growth.

Upton fails to disclose, or provide any rational basis for, a cell culture medium that (i) comprises a synthetic IGF/VN fragment chimeric protein that does not comprise an IGFBP, and (ii) is suitable for cell culture in the absence of serum. Upon reading Upton, one skilled in the art would be more likely to conclude, if anything, that serum was temporarily excluded from the assay medium to avoid the potentially complicating interference of serum proteins (*e.g.*, growth factors) during the assay. Moreover, Upton does not provide any rational basis for removal of poorly defined, animal-derived exogenous factors (*e.g.*, serum) from the medium for the purposes of therapeutic treatments that require skin growth *ex vivo* (*see*, *e.g.*, p. 2, ll. 16-22).

Significantly, the presently claimed invention stems from the inventors' unexpected discovery that cell culture media comprising synthetic IFN/VN fragment chimeras are capable of stimulating cell migration and growth in the absence of an IGFBP in no or low serum. This discovery is associated with significant advantages. For example, instead of producing a multiprotein complex consisting of an IGFBP, full-length VN, and an IGF, a small bioactive protein (*i.e.*, an IGF-VN fragment synthetic chimera) can replicate the action of the multiprotein complex and support cell migration and proliferation in the absence of serum. Advantageously, elimination

of the "bulky" IGFBP portion and truncation of the VN molecule to only include essential features also translates into improved production efficiencies and reduced development costs.

Moreover, neither Upton nor any of the other references cited in the instant rejection teach or provide a rational basis for a serum-free cell culture medium comprising synthetic IGF/VN fragment chimeric proteins would have sustained epithelial cell growth in the absence of an IGFBP because, prior to the present invention, combinations of IGF-II and VN were always grown in the presence of 5-10% serum, while IGF-I was always grown in the presence of an IGFBP and 5-10% serum.

Accordingly, Applicants respectively submit that the inventors' discovery that synthetic chimeric proteins comprising (i) an IGF-I or an IGF-II, and (ii) an α_v integrin-binding VN domain that does not comprise a HBD can support essentially serum-free, long-term cultivation of epithelial cells in the absence of an IGFBP is a surprising and unexpected result that is non-obvious in view of Upton. In other words, one of ordinary skill in the art at the time of the present invention would not have designed a serum-free mammalian cell culture medium comprising a synthetic IGF/VN fragment chimeric protein that does not comprise an IGFBP with a reasonable expectation of success because the prior art teaches that serum, at least an IGFBP, and full-length VN are required for optimum cell migration and proliferation.

II. Vuori

The Office Action argues that Vuori supplements the teachings of Upton by teaching a recombinant complex comprising the growth factor IGF-I, an IGFBP3 and VN, and a pharmaceutical composition comprising the isolated protein complex.

Applicants respectively submit that Vuori merely teaches a biodegradable polymer matrix that forms a conjugate with an integrin ligand and growth factor "dispersed within" the matrix. This is expressed as "incorporated within a matrix" in Claim 1 of Vuori; however, Applicants point out that this is not a suggestion that IGF-I forms a complex with VN. Rather, it is merely a suggestion that a growth factor (*i.e.*, not specifically IGF-I) might be dispersed within a matrix that can form a conjugate with an integrin ligand (*i.e.*, not specifically VN).

It should be appreciated that a complex is different than a dispersion. A complex comprises two or more molecules held together in a definable structure relationship as a result of a specific interaction. The terms "dispersion" and "incorporation" as used by Vuori relate to containment of a growth factor within a polymer matrix. There is no teaching or basis in Vuori that the growth factor has formed a complex with the matrix, let alone with the integrin ligand (*e.g.*, VN). Vuori refers to conjugation of the integrin ligand to the matrix, but no such teaching is made with respect to the growth factor, as is clear from Claim 2, for example. The growth factor (*e.g.*, VN) is simply not part of a protein complex in Vuori. Thus, Vuori does not teach or provide a basis for an isolated protein <u>complex</u> comprising a growth factor and an integrin ligand.

III. Sommer

Sommer only teaches protein complexes comprising an IGF and an IGFBP, and their potential role in wound and burn healing. Sommer is specifically concerned with the IGF-I/IGFBP complex *per se*, and there is no rational basis therein that it might be useful to consider the use of additional biologically-active molecules to form a complex with the IGF-I/IGFBP, let alone a molecule like VN. The only teaching relevant to additional molecules is in the section at Col. 8, ll. 9-47, which relates to various solvents, diluents, and polymeric carriers (*e.g.*, suppositories and microcapsules for sustained release).

Sommer actually teaches a complex comprising IGF-I and an IGFBP. At Col. 4, ll. 54-58, for example, Sommer states that "[w]hile not wishing to be bound by any particular theory, the inventors propose that the systemically administered IGF and IGFBP composition first raises the blood level of IGF/IGFBP. The IGF is then carried to the regenerating tissues via the circulation." Thus, in contrast to the present invention, which claims a serum-free cell culture medium comprising chimeric IGF/VN fragment proteins that do not include an IGFBP, Sommer teaches that it is systemic administration of IGF/IGFBP complexes that promotes the biological activity of the complex in relation to wound repair and tissue regeneration via the blood.

As will be appreciated from the foregoing, Sommer, Vuori, and Upton actually teach away from the present invention as these references teach: (i) a serum-containing medium or, at most, a serum-free assay medium that would not be suitable for cell culture; (ii) the presence of an IGFBP; and (iii) full-length VN. Thus,

there would be no reasonable expectation that a serum-free medium comprising synthetic IGF/VN fragment chimeras would be able to successfully support cell migration and proliferation in the absence of an IGFBP.

IV. Klemke

Klemke teaches that receptor tyrosine kinase signaling is required for integrin $\alpha_v\beta5$-directed FG pancreatic cell motility, but not adhesion to full-length VN. In contrast to Klemke, which teaches the use of full-length VN (*see*, *e.g.*, p. 860, 1st column, "Adhesive Ligands" section) and Yatohgo *et al.*, *Cell Structure and Function* 13:281-292, 1988 (hereinafter, "Yatohgo") (included herewith), the inventors of the present invention unexpectedly discovered that synthetic proteins comprising short VN fragments (*e.g.*, lacking the HBD) can bind the extracellular matrix (ECM) and thereby provide a matrix for cell attachment and migration in the absence of an IGFBP. Given that Yatohgo refers to VN purified by heparin-sepharose chromatography, it is clear that the VN described in Yatohgo, and used by Klemke, included the HBD.

Moreover, there is nothing in Klemke, alone or in combination with any of the cited references, that teaches or provides a basis for a mammalian cell culture medium comprising synthetic chimeric proteins that comprise (i) an IGF-I or an IGF-II, and (ii) an $\alpha_v$integrin-receptor binding VN fragment that lacks the HBD, wherein the medium is capable of supporting long-term cell growth in the absence of an IGFBP and in low or no serum.

A. Hocking et al., J Biol Chem. 274(38):27257-27264, 1999 (hereinafter, "Hocking")

Hocking teaches that the HBD of VN regulates the deposition of fibronectin into the extracellular matrix through alternations in the organization of the actin cytoskeleton. Hocking provides no rational basis, however, for one of ordinary skill in the art at the time of the present invention to have combined Hocking with Klemke to (i) create a small biologically active VN fragment by removing the HBD from VN, (ii) produce a synthetic IGF/VN fragment chimeric protein that does not comprise an IGFBP, and (iii) use the synthetic chimeric protein in a serum-free cell culture medium to stimulate cell migration and growth and promote wound healing *in vivo*.

V. Nam

Applicants respectively submit that one skilled in the art at the time of the present invention would not have had a rational basis to combine Nam with the other cited references because: (1) Nam teaches the use of a cell culture medium that includes an IGFBP; and (2) there is no reason that one skilled in the art would modify the short-term endothelial assay medium of Nam by (i) removing the IGFBP, and/or (ii) producing synthetic chimeras comprising an IGF and a VN fragment as a means of optimizing mammalian cell culture in low or no serum.

Nam discloses a study in which interactions between IGFBP5, VN, and IGF-I, as well as the effects these molecules have on cell migration and DNA synthesis in smooth muscle cells (*i.e.*, endothelial cells) using short-term assays were investigated. Nam does not teach or disclose, however, that the endothelial cell migration assay medium, which included ^{125}I-IGFBP5, was an optimal cell culture

medium. Additionally, the teachings of Nam are not relevant to the challenge of culturing graftable epithelial cells (*e.g.*, keratinocytes and/or corneal cells) in a way that minimizes the use of poorly characterized exogenous factors, such as serum that can contaminate cultured cells with pathogens and thereby pose a health risk when the cultured cells are used for skin grafting and like treatments. Moreover, Nam fails to teach or provide any basis for methods to optimize cell culture.

Unlike the teachings of Nam, the claims of the present invention are directed to an essentially serum-free mammalian epithelial cell culture medium comprising a synthetic IGF/VN fragment chimeric protein that does not comprise an IGFBP. There is simply no rational basis in Nam that would have led one skilled in the art at the time of the present invention to modify the short-term endothelial assay medium of Nam by (i) removing the IGFBP, and/or (ii) producing synthetic chimeras comprising an IGF and a VN fragment as a means of optimizing mammalian cell culture in low or no serum.

VI. Conclusion.

The present invention is based on the unexpected and surprising discovery that an essentially serum-free mammalian cell culture medium comprising synthetic IGF/VN fragment chimeric proteins is able to support long-term epithelial cell growth and expansion in the absence of an IGFBP. There is nothing in the cited references that teaches or provides a basis for a cell culture medium comprising synthetic IGF/VN fragment chimeric proteins capable of sustaining epithelial cell growth in the absence of an IGFBP and serum. Prior to the present invention, for example, combinations of IGF-II and VN were always grown in the presence of 5-10% serum,

while IGF-I and VN were always grown in the presence of at least an IGFBP and 5-10% serum. One skilled in the art at the time of the present invention would simply not have designed a cell culture medium that excludes (i) serum, (ii) an IGFBP, and (iii) the HBD of VN because the references cited in the instant rejection teach that these components are essential to support cell growth and migration.

Accordingly, Applicants respectively submit that amended claim 1 is patentable over Upton, Vuori, and Sommer in view of Klemke and Nam, and request that the 35 U.S.C. §103(a) rejection of claim 1 be withdrawn. Additionally, Applicants respectively request that the 35 U.S.C. §103(a) rejection of claims 2, 5, 7, 21-23 and 37-38, which depend either directly or indirectly from amended claim 1, be withdrawn.

Please charge any deficiency or credit any overpayment in the fees for this matter to our Deposit Account No. 20-0090.

Respectfully submitted,

/Richard S. Wesorick/

Richard S. Wesorick
Reg. No. 40,871

TAROLLI, SUNDHEIM, COVELL,
& TUMMINO L.L.P.
1300 East Ninth Street, Suite 1700
Cleveland, Ohio 44114
Phone: (216) 621-2234
Fax: (216) 621-4072
Customer No.: 26,294